Risk Stratification of Patients with Stage I Cutaneous Melanoma Using 31-Gene Expression Profiling

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ABSTRACT

BACKGROUND: While patients with localized cutaneous melanoma (CM) generally have good five-year melanoma-specific survival rates, identifying patients with localized disease at a high risk of recurrence could allow them access to additional follow-up or surveillance. **OBJECTIVE:** We sought to examine the prognostic value of the 31-gene expression profile (31-GEP) test for the risk of recurrence in stage I CM patients according to 31-GEP main class (low risk: Class 1 vs. high-risk: Class 2) and the lowest and highest risk 31-GEP subclasses (Class 1A vs. Class 2B). **METHODS:** Data from a previously described meta-analysis detailing the 31-GEP results for patients with stage I CM (N = 623) were re-analyzed to determine 31-GEP accuracy. **RESULTS:** Patients with stage I CM and a Class 1 31-GEP result were less likely to have a recurrence (15/556; 2.7% vs. 6/67; 9.0%; p=0.018) than patients with a Class 2 result and had a higher five-year recurrence-free survival (RFS) (96% vs. 85%). Patients with a Class 2 result were 2.8 times as likely to experience a recurrence (positive likelihood ratio: 2.82; 95% confidence interval: 1.38–5.77). In a subset of patients with stage I CM stratified further into 31-GEP subclasses (n = 206), patients with a Class 1A result had a higher five-year RFS than those with a Class 2B result (98% vs. 73%). Patients with a Class 2B result were also 6.5 times as likely to experience a recurrence (positive likelihood ratio: 6.45; 95% confidence interval: 2.44–17.00) than those with a Class 1A result, and the 31-GEP had a negative predictive value of 96.3% (95% confidence interval: 92.3%–98.4%). **CONCLUSIONS:** The 31-GEP test significantly differentiates between low and high recurrence risk in patients with stage I CM.

KEY WORDS: 31-gene expression profile, cutaneous melanoma, American Joint Committee on Cancer, stage I cutaneous melanoma

While patients with localized cutaneous melanoma (CM) generally have good five-year melanoma-specific survival rates (stage I, 98%; stage II, 90%) according to the eighth edition of the American Joint Committee on Cancer staging system (AJCC8), the five-year recurrence-free survival (RFS) for this population is rarely reported for stage I and can fall as low as 85%, while the five-year RFS for stage II can range from 59% to 76%, with national guidelines recognizing the increased risk with stage IIB and IIC localized disease.[1–3] Identifying patients with localized disease at a high risk of recurrence could allow these patients access to additional follow-up or surveillance. In contrast, patients with a low recurrence risk may be able to forego costly or risky therapies.[4] The 31-gene expression profile (31-GEP) test compares RNA levels from primary tumors using quantitative real-time polymerase chain reaction, employing 28 discriminating and three control probes. It assigns a relative risk from a radial basis machine learning algorithm to derive low risk (Class 1A), intermediate-risk (Classes 1B and 2A), or high-risk (Class 2B) results.[5,6]

Multiple studies have suggested the significant and independent prognostic value of the 31-GEP in patients with stage I to III CM.[5–7] However, a recent study by Marchetti et al. suggested that, while the 31-GEP's ability to provide an accurate prognosis for patients diagnosed with stage II disease is useful, data supporting the use of the 31-GEP in stage I CM are lacking.[8] However, the analyses performed and conclusions posited by Marchetti et al. failed to provide a complete picture of 31-GEP testing for stage I CM. Among the methodological limitations of the Marchetti et al. study is the lack of a thorough analysis. Because Marchetti and colleagues did not assess the raw data, they did not have enough information to conduct a multivariable analysis nor could they assess the more clinically used lowest- (Class 1A) and highest-risk (Class 2B) results. However, a comparison of Classes 1 and 2 using diagnostic odds (DOR) or positive and negative likelihood ratios could have helped the authors to make a more informed conclusion on the stratification of risk within the stage I population. Because Marchetti et al. presented limited analyses of 31-GEP testing in patients with stage I melanoma, we re-analyzed data for the 623 patients with stage I CM reported by Marchetti to show how the 31-GEP can refine recurrence risk assessment for patients with stage I CM.

First, Marchetti et al. accurately stated that most patients (82%) with stage II melanoma who experienced a recurrence were correctly classified as being at higher risk (Class 2) and that 90% of patients with stage I melanoma who did not have a recurrence were correctly classified as being at low risk (Class 1). Of patients with stage I CM and a Class 1 31-GEP result, just 2.7% (15/556) experienced a recurrence. Further, the authors are correct that, in a combined stage I–II analysis with the 31-GEP, most events occur in stage II melanoma and most nonevents occur in stage I melanoma, as expected. However, Marchetti et al. failed to report that, within each AJCC stage, patients with a Class 2 result consistently have significantly lower five-year RFS than those

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TABLE 1. Summary of 5-year RFS accuracy (95% CI) using 31-GEP Classes 1 and 2 for patients with stage I CM from the study of Marchetti et al.[8]

31-GEP RESULT	FIVE-YEAR RFS	RECURRENCE	NO RECURRENCE	SENSITIVITY†	SPECIFICITY†	PPV†	NPV†	POSITIVE LIKELIHOOD RATIO	NEGATIVE LIKELIHOOD RATIO	DOR
Class 1	96%	15	541	28.6% (12.2%–52.3%)	89.9% (87.1%–92.1%)	9.0% (3.7%–19.1%)	97.3% (95.5%–98.4%)	2.82 (1.38–5.77)	0.79 (0.61–1.04)	3.55
Class 2	85%	6	61							

†Surrogate measures as these metrics are best determined at a specific time point.
RFS: recurrence-free survival; PPV: positive predictive value; NPV: negative predictive value; DOR: diagnostic odds ratio [(TP/FN)/(FP/TN)].

TABLE 2. Summary of 5-year RFS accuracy (95% CI) using 31-GEP Classes 1A and 2B for patients with stage I CM from the study of Zager et al.[6]

31-GEP RESULT	FIVE-YEAR RFS	RECURRENCE	NO RECURRENCE	SENSITIVITY	SPECIFICITY	PPV	NPV	POSITIVE LIKELIHOOD RATIO	NEGATIVE LIKELIHOOD RATIO	DOR
Class 1A	98%	7	184	36.4% (12.4%–68.4%)	94.4% (89.9%–97.0%)	26.7% (8.9%–55.2%)	96.3% (92.3%–98.4%)	6.45 (2.44–17.00)	0.67 (0.43–1.05)	9.56
Class 2B	73%	4	11							

RFS: recurrence-free survival; PPV: positive predictive value; NPV: negative predictive values; DOR: diagnostic odds ratio [(TP/FN)/(FP/TN)].

with a Class 1 result, thus further stratifying the patient recurrence risk (Table 1). The authors appear to assume that clinicians use the 31-GEP test results to make clinical decisions irrespective of clinicopathologic factors. However, clinicians are trained to integrate diverse data types to guide decision-making, and GEP testing in melanoma is supported by evidence that genetic information derived from the tumor can refine—not replace—staging. For example, the 31-GEP class can be combined with the clinical stage to guide surveillance plans, and a recent meta-analysis has demonstrated that the 31-GEP test improves the risk assessment when incorporated together with the patient's AJCC8 stage.[9,10]

Additional analyses of the data show that—counter to Marchetti et al.'s. conclusion that the 31-GEP does not improve risk stratification in stage I CM—a Class 2 result had a DOR of 3.55, and patients with a Class 2 result were 2.8 times as likely to experience a recurrence than those with a Class 1 result [positive likelihood ratio: 2.82; 95% confidence interval (CI): 1.38–5.77] (Table 1), indicating that a 31-GEP Class 2 result is associated with an approximately three-fold increase in recurrence as compared with a Class 1 result in the stage I population.

Additional analysis of the subset of cases, in which the more clinically used lowest-risk (Class 1A) and highest-risk (Class 2B) results were published (n = 206), revealed an even greater increase in recurrence risk for patients with a Class 2B result.[6] Patients with a Class 1A 31-GEP result had a higher five-year RFS than patients with a Class 2B result (98% vs. 73%) and a DOR of 9.56. Patients with a Class 2B result were 6.5 times as likely to experience a recurrence than those with a Class 1A result (positive likelihood ratio: 6.45; 95% CI: 2.44–17.00). Moreover, a Class 1A result had a high negative predictive value of 96.3% (Table 2). These data show that the 31-GEP test significantly separates patients with stage I CM into groups at high and low risk of recurrence, respectively. Because Marchetti et al. only reported on 31-GEP main classes (Class 1 vs. Class 2), they likely missed much of the value of the 31-GEP derived from separating the lowest-risk patients (Class 1A) from the highest-risk patients (Class 2B), which may be more pronounced in stage I melanoma.

Moreover, the study by Marchetti et al. had multiple limitations, resulting in misrepresentation and biases against the 31-GEP. The criteria for bias detection (QUIPS) were applied inconsistently throughout studies as negative features in one study (e.g., retrospective, unclear sampling size determination) are not reported as positive features when the opposite is true for another study (e.g., prospective, or clear sample size determination). Finally, the authors requested comparisons to "multivariable risk-prediction models" not included in guidelines. The 31-GEP has demonstrated independent and significant RFS prediction when analyzed in comparison with multiple clinicopathologic features.[5–7] The fact that other prognostic tools were not held to the same standard raises questions of bias.

In summary, the metrics reported by Marchetti et al. are numerically correct but fail to appreciate the clinical utility of the 31-GEP test. Prognostic tests rarely have perfect accuracy and instead modify the event probability of a case. Further, the estimated proportions reported by Marchetti et al. did not capture the relative change in diagnostic accuracy that describes an event's relative likelihood between two groups. Finally, as the DOR and positive likelihood ratio could have easily been calculated from the data available to them, we have chosen to conclude that Marchetti and colleagues were not aware of these metrics or their utility rather than accepting the alternative conclusion that they presented a deliberately flawed analysis to advance their thesis.

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